

What is claimed is:

1. A method of collecting data for estimating susceptibility to periodontal disease, wherein the method comprises:

5 in order to detect the presence of a gene mutation and/or a mutation site existing in the promoter region of a human defensin gene in a sample,

by using a nucleotide sequence being a part of the promoter of the defensin gene and comprising a mutation site, as a
10 nucleotide sequence for a probe,

determining

(i) a hybridization site of hybridization between the defensin gene promoter nucleotide sequence in the sample and said probe, and/or

15 (ii) an amplification ability in gene amplification where primers comprising the nucleotide sequence of said probe are used;

thereby clarifying the change of the activity of the defensin promoter to regulate the expression of the defensin
20 gene based on the thus detected presence of a gene mutation and/or a mutation site.

2. A method of collecting data for estimating susceptibility to periodontal disease, wherein the method comprises:

in order to detect the presence of a gene mutation and/or a mutation site existing in the promoter region of a human β -defensin 2 gene in a sample,

by using a nucleotide sequence being a part of the promoter
5 of the β -defensin 2 gene and comprising a mutant nucleotide, as a nucleotide sequence for a probe,

determining

(i) a hybridization site of hybridization between the β -defensin 2 gene promoter nucleotide sequence in the sample
10 and said probe, and/or

(ii) an amplification ability in gene amplification where primers comprising the nucleotide sequence of said probe are used;

thereby clarifying the change of the activity of the human
15 β -defensin 2 promoter to regulate the expression of the β -defensin 2 gene based on the thus detected presence of a gene mutation and/or a mutation site.

3. A nucleotide sequence used as a probe to obtain data for
20 estimating susceptibility to periodontal disease, wherein the nucleotide sequence is used to detect a mutant type sequence existing in the promoter region of a human β -defensin 2 gene, and comprises at least 5 nucleotides being each of upstream and downstream from a mutation site, otherwise

at-least-10-nucleotide-containing sequences of which 3' terminus is the nucleotide of a mutation site in the promoter nucleotide sequences.

- 5 4. The nucleotide sequence used as a probe according to claim 3, wherein said nucleotide sequence is any sequence selected from:

a DNA nucleotide sequence amplified by primer set 1:

5' ATAGGCGTAAGCCATCATGCC 3' (SEQ ID NO:1)

10 5' CATCCTGGTTCCTCCCTCTTT 3' (SEQ ID NO:2)

wherein G is substituted by C at a site -1431 located upstream of the transcription initiation point of the human β -defensin 2 gene, and/or

a DNA nucleotide sequence amplified by primer set 2:

15 5' TGTTTCTCAAAGTCCCTTAG 3' (SEQ ID NO:3)

5' ATGGGATTGTGACTACATGTG 3' (SEQ ID NO:4)

wherein G is substituted by T at a site -1035 (mutation site 2-1), and/or A is substituted by G at a site -1027 (mutation site 2-2), and/or G is substituted by A at a site -936 (mutation site 2-3), and/or C is substituted by T at a site -923 (mutation site 2-4),

and/or a DNA nucleotide sequence amplified by the same primer set as above, wherein T is substituted by C at a site -912

(mutation site 2-5), and/or G is substituted by A at a site -874 (mutation site 2-6), and/or

a DNA nucleotide sequence amplified by primer set 3:

5' TCCGGACCCACTTGAGACTCC 3' (SEQ ID NO:5)

5 5' GAAAATTCCTCCTATCTTGCA 3' (SEQ ID NO:6)

wherein C is substituted by T at a site -539 (mutation site 3-1), and/or A is substituted by G at a site -472 (mutation site 3-2), and/or

a DNA nucleotide sequence amplified by primer set 4:

10 5' ACTCCATTACACACTGGGTT 3' (SEQ ID NO:7)

5' AACGAGAAGAGGAGATACAAG 3' (SEQ ID NO:8)

wherein T is substituted by C at a site -108 (mutation site 4).

15 5. The nucleotide sequence used as a probe according to claim 3, wherein said nucleotide sequence is further modified with markers for detection and/or amplification.

6. A primer which comprises both nucleotide sequences of
20 primer set 1:

5' ATAGGCGTAAGCCATCATGCC 3' (SEQ ID NO:1)

5' CATCCTGGTTCCTCCCTCTTT 3' (SEQ ID NO:2)

and is used to amplify DNA derived from a human defensin gene.

7. A primer which comprises both nucleotide sequences of primer set 2:

5' TGTTTCTCAAAGTGGCCCTTAG 3' (SEQ ID NO:3)

5' ATGGGATTGTGACTACATGTG 3' (SEQ ID NO:4)

5 and is used to amplify DNA derived from a human defensin gene.

8. A primer which comprises both nucleotide sequences of primer set 3:

5' TCCGGACCCACTTGAGACTCC 3' (SEQ ID NO:5)

10 5' GAAAATTCCTCCTATCTTGCA 3' (SEQ ID NO:6)

and is used to amplify DNA derived from a human defensin gene.

9. A primer which comprises both nucleotide sequences of primer set 4:

15 5' ACTCCATTCACACACTGGGTT 3' (SEQ ID NO:7)

5' AACGAGAAGAGGAGATACAAG 3' (SEQ ID NO:8)

and is used to amplify DNA derived from a human defensin gene.

10. A primer which has any one of the nucleotide sequences
20 used as probes according to claim 4, and is used to determine
an amplification ability in gene amplification.

11. The nucleotide sequence used as a probe according to claim 4, wherein said nucleotide sequence is further modified with markers for detection and /or amplification.

5 12. A kit used to estimate susceptibility to periodontal disease wherein the kit comprises at least one type of a probe comprising a nucleotide sequence to detect a mutant type sequence existing in the promoter region of a human defensin
2 gene, and optionally comprising a primer such as at least
10 5 nucleotides being each of upstream and downstream from a mutation site and otherwise at-least-10-nucleotide-containing sequences of which 3' terminus is the nucleotide of a mutation site in the promoter nucleotide sequences, so as to detect a mutant gene existing in the promoter region
15 of a human defensin gene.

13. The kit according to claim 12, wherein the nucleotide sequence used as a probe is at least one sequence selected from:

20 a DNA nucleotide sequence amplified by primer set 1:

5' ATAGGCGTAAGCCATCATGCC 3' (SEQ ID NO:1)

5' CATCCTGGTTCCTCCCTCTTT 3' (SEQ ID NO:2)

wherein G is substituted by C at a site -1431 located upstream of the transcription initiation point of the human β -defensin 2 gene, and/or

a DNA nucleotide sequence amplified by primer set 2:

5 5' TGTTTCTCAAACCTGCCCTTAG 3' (SEQ ID NO:3)

5' ATGGGATTGTGACTACATGTG 3' (SEQ ID NO:4)

wherein G is substituted by T at a site -1035 (mutation site 2-1), and/or A is substituted by G at a site -1027 (mutation site 2-2), and/or G is substituted by A at a site -936 (mutation site 2-3), and/or C is substituted by T at a site -923 (mutation site 2-4), and/or

a DNA nucleotide sequence amplified by the same primer set as above, wherein T is substituted by C at a site -912 (mutation site 2-5), and/or G is substituted by A at a site -874 (mutation site 2-6), and/or

a DNA nucleotide sequence amplified by primer set 3:

5' TCCGGACCCACTTGAGACTCC 3' (SEQ ID NO:5)

5' GAAAATTCCTCCTATCTTGCA 3' (SEQ ID NO:6)

wherein C is substituted by T at a site -539 (mutation site 3-1), and/or A is substituted by G at a site -472 (mutation site 3-2), and/or

a DNA nucleotide sequence amplified by primer set 4:

5' ACTCCATTACACACTGGGTT 3' (SEQ ID NO:7)

5' AACGAGAAGAGGAGATACAAG 3' (SEQ ID NO:8)

wherein T is substituted by C at a site -108 (mutation site 4).

14. The kit according to claim 12, further comprising at least
5 one primer selected from:

a primer which comprises both nucleotide sequences of
primer set 1:

5' ATAGGCGTAAGCCATCATGCC 3' (SEQ ID NO:1)

5' CATCCTGGTTCCTCCCTCTTT 3' (SEQ ID NO:2)

10 and is used to amplify a DNA derived from a human defensin
gene;

a primer which comprises both nucleotide sequences of
primer set 2:

5' TGTTTCTCAAAGTGCCTTAG 3' (SEQ ID NO:3)

15 5' ATGGGATTGTGACTACATGTG 3' (SEQ ID NO:4)

and is used to amplify a DNA derived from a human defensin
gene;

a primer which comprises both nucleotide sequences of
primer set 3:

20 5' TCCGGACCCACTTGAGACTCC 3' (SEQ ID NO:5)

5' GAAAATTCCTCCTATCTTGCA 3' (SEQ ID NO:6)

and is used to amplify a DNA derived from a human defensin
gene;

a primer which comprises both nucleotide sequences of primer set 4:

5' ACTCCATTACACACTGGGTT 3' (SEQ ID NO:7)

5' AACGAGAAGAGGAGATACAAG 3' (SEQ ID NO:8)

5 and is used to amplify a DNA derived from a human defensin gene.

15. A DNA chip wherein the DNA chip comprises at least one type of a probe comprising a nucleotide sequence to detect
10 a mutant type sequence existing in the promoter region of a human defensin 2 gene, and optionally comprising a primer such as at least 5 nucleotides being each of upstream and downstream from a mutation site and otherwise at-least-10-nucleotide-containing sequences of which 3' terminus is the nucleotide
15 of a mutation site in the promoter nucleotide sequences, so as to detect a mutant gene existing in the promoter region of a human defensin gene.

16. The DNA chip according to claim 15, wherein the nucleotide
20 sequence used as a probe is at least one sequence selected from:

a DNA nucleotide sequence amplified by primer set 1:

5' ATAGGCGTAAGCCATCATGCC 3' (SEQ ID NO:1)

5' CATCCTGGTTCCTCCCTCTTT 3' (SEQ ID NO:2)

wherein G is substituted by C at a site -1431 located upstream of the transcription initiation point of the human β -defensin 2 gene, and/or

a DNA nucleotide sequence amplified by primer set 2:

5 5' TGTTTCTCAAACCTGCCCTTAG 3' (SEQ ID NO:3)

5' ATGGGATTGTGACTACATGTG 3' (SEQ ID NO:4)

wherein G is substituted by T at a site -1035 (mutation site 2-1), and/or A is substituted by G at a site -1027 (mutation site 2-2), and/or G is substituted by A at a site -936 (mutation site 2-3), and/or C is substituted by T at a site -923 (mutation site 2-4), and/or

a DNA nucleotide sequence amplified by the same primer set as above, wherein T is substituted by C at a site -912 (mutation site 2-5), and/or G is substituted by A at a site -874 (mutation site 2-6), and/or

a DNA nucleotide sequence amplified by primer set 3:

5' TCCGGACCCACTTGAGACTCC 3' (SEQ ID NO:5)

5' GAAAATTCCTCCTATCTTGCA 3' (SEQ ID NO:6)

wherein C is substituted by T at a site -539 (mutation site 3-1), and/or A is substituted by G at a site -472 (mutation site 3-2), and/or

a DNA nucleotide sequence amplified by primer set 4:

5' ACTCCATTACACACTGGGTT 3' (SEQ ID NO:7)

5' AACGAGAAGAGGAGATACAAG 3' (SEQ ID NO:8)

wherein T is substituted by C at a site -108 (mutation site 4).

17. The DNA chip according to claim 15, further comprising
5 at least one primer selected from:

a primer which comprises both nucleotide sequences of primer set 1:

5' ATAGGCGTAAGCCATCATGCC 3' (SEQ ID NO:1)

5' CATCCTGGTTCCTCCCTCTTT 3' (SEQ ID NO:2)

10 and is used to amplify a DNA derived from a human defensin gene;

a primer which comprises both nucleotide sequences of primer set 2:

5' TGTTTCTCAAAGTGCCTTAG 3' (SEQ ID NO:3)

15 5' ATGGGATTGTGACTACATGTG 3' (SEQ ID NO:4)

and is used to amplify a DNA derived from a human defensin gene;

a primer which comprises both nucleotide sequences of primer set 3:

20 5' TCCGGACCCACTTGAGACTCC 3' (SEQ ID NO:5)

5' GAAAATTCCTCCTATCTTGCA 3' (SEQ ID NO:6)

and is used to amplify a DNA derived from a human defensin gene;

a primer which comprises both nucleotide sequences of primer set 4:

5' ACTCCATTACACACTGGGTT 3' (SEQ ID NO:7)

5' AACGAGAAGAGGAGATACAAG 3' (SEQ ID NO:8)

5 and is used to amplify a DNA derived from a human defensin gene.

18. A method for estimating susceptibility to periodontal disease on the basis of nucleotide sequences revealed by a
10 human allele analysis, using the allele specific PCR method.